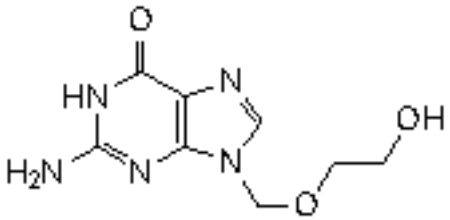
Aciclovir (Aciclovirum)

2014-01



Molecular formula. $C_8H_{11}N_5O_3$

Relative molecular mass. 225.20

Chemical name. 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one; 9-[(2-hydroxyethoxy)methyl]guanine; CAS Reg. No. 59277-89-3.

Description. White or almost white, crystalline powder.

Solubility. Slightly soluble in water; freely soluble in dimethyl sulfoxide; very slightly soluble in ethanol (96%). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

Category. Antiviral (Purine nucleoside analogue).

Storage. Preserve in well-closed containers. Protect from light and moisture.

Additional information. Aciclovir may exhibit polymorphism.

Requirements

Definition. Aciclovir contains not less than 98.5% and not more than 101.0% of $C_8H_{11}N_5O_3$, calculated with reference to the dried substance.

Identity tests

-Either test A alone, or test B and D or test C and D may be applied.

A. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS or with the *reference spectrum* of aciclovir. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and aciclovir RS in a small amount of hot water R and evaporating on a water-bath to dryness. Dry the residues at 100–105 °C for 3 h. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS.

B. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using the conditions given under Guanine and “Related substances”, Test A. The principal spot in the chromatogram obtained with solution (B) corresponds in position, appearance and intensity to the spot due to aciclovir in the chromatogram obtained with solution (C).

C. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given under Guanine and “Related substances”, Test B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to aciclovir in the chromatogram obtained with solution (4).

D. Dissolve about 10 mg of the test substance in 5.0 mL of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 mL with water R. Dilute 5.0 mL of this solution to 50.0 mL with water R. The absorption spectrum (*1.6*) of the resulting solution, when observed between 230 nm and 350 nm, exhibits one maximum at about 255 nm; the specific absorbance ($A^{1\%}_{1cm}$) at 255 nm is about 500.

Clarity and colour of solution. A solution, containing 0.25 g of the test substance in 25 mL of sodium hydroxide (0.1 mol/L) TS, is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under *1.11.1 Colour of liquids*.

Sulfated ash (2.3). Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 60 mg/g.

Guanine and related substances

-Either test A or test B may be applied.

A. **Guanine.** Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using cellulose R1 as the coating substance and a mixture of 10 volumes of propan-1-ol, 30 volumes of ammonia (260 g/l) TS and 60 volumes of ammonium sulfate (50 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following four, freshly prepared solutions in sodium hydroxide (0.1 mol/l) TS. For solution (A) use 5 mg of the test substance per mL. For solution (B) dilute 1 volume of solution (A) to 10 volumes. For solution (C) use a solution of 0.5 mg of aciclovir RS and 0.5 mg of guanine R per mL. For solution (D) use 35 µg of guanine R per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). In the chromatogram obtained with solution (C) guanine is eluted with a R_f value of 0.5 and aciclovir with a R_f value of 0.7. The test is not valid unless this chromatogram shows two clearly separated spots. Any secondary spot corresponding to guanine in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (0.7%).

B. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 1 volume of acetonitrile R and 99 volumes of phosphate buffer, pH 3.1, TS.

Mobile phase B: 50 volumes of acetonitrile R and 50 volumes of phosphate buffer, pH 2.5, TS.

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comments
0–5	100	0	Isocratic
5–27	100→80	0→20	Linear gradient
27–40	80	20	Isocratic
40–42	80→100	20→0	Return to initial composition
42–52	100	0	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) dissolve 25 mg of the test substance in 5.0 mL of sodium hydroxide (0.1 mol/l) TS and dilute to 25.0 mL with water R. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with water R. Dilute 1.0 mL of this solution to 10.0 mL with water R. For solution (3) dissolve 10 mg of guanine R in 10 mL of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 mL with water. Dilute 5.0 mL of this solution to 50.0 mL with water R. For solution (4) dissolve 5 mg of aciclovir RS, 5 mg of guanine R and 10 mg of aciclovir impurity C RS in 10 mL of sodium hydroxide (0.1 mol/l) TS and dilute to 100 mL with water R.

Inject separately 20 µl each of solutions (1), (2), (3) and (4).

The following peaks are eluted at the following relative retention with reference to the peak of aciclovir (retention time about 13 min): impurity B about 0.4; impurity P about 0.7; impurity C about 0.9; impurity I about 1.57; impurity J about 1.62; impurity F about 1.7; impurity A about 1.8; impurity K about 2.5 and impurity G about 2.6.

The test is not valid unless in the chromatogram obtained with solution (4) the resolution factor between the peak due to aciclovir impurity C and the peak due to aciclovir is at least 1.5.

In the chromatogram obtained with solution (1):

· the area of any peak corresponding to guanine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.7 %);

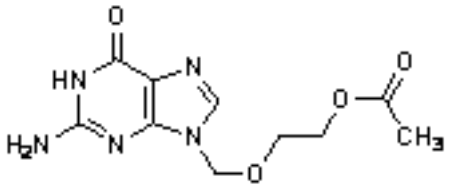
· the area of any other peak, other than the principal peak, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

· the sum of all other areas, other than the principal peak and the peak due to guanine, is not greater than 10 times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.03%).

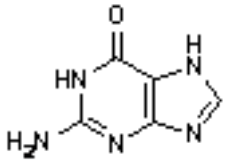
Assay

Dissolve about 0.150 g, accurately weighed, in 60 mL of anhydrous acetic acid R. Titrate with perchloric acid (0.1mol/l) VS, determining the end-point potentiometrically as described under *2.6 Non-aqueous titration*. Carry out a blank titration. Each mL of perchloric acid (0.1 mol/l) VS is equivalent to 22.52 mg of aciclovir ($C_8H_{11}N_5O_3$).

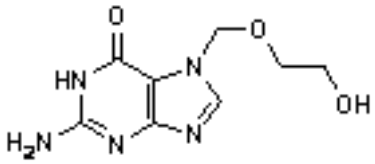
Impurities



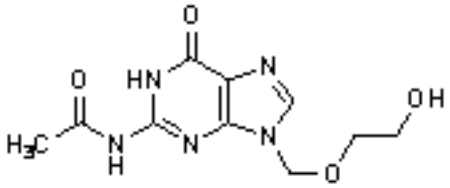
A. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,



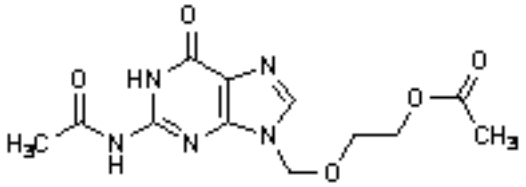
B. 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine),



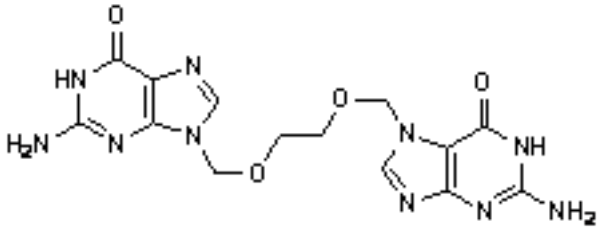
C. 2-amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one,



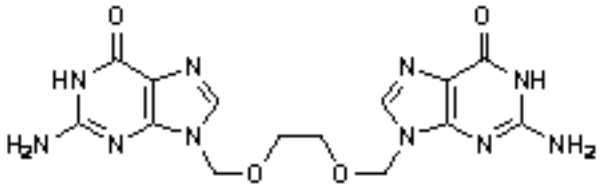
F. *N*-{9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}acetamide (N^2-acetylaciclovir),



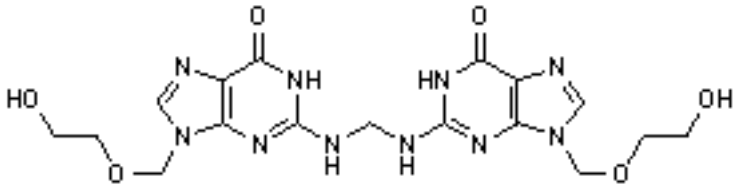
G. 2-[(2-acetamido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,



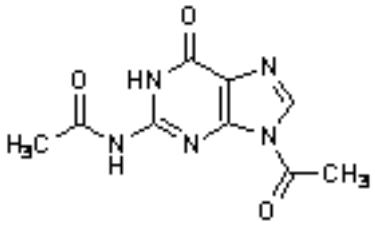
I. 2-amino-7-({[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethoxy}methyl)-1,7-dihydro-6*H*-purin-6-one (7,9'-[ethylenebis(oxymethylene)]diguanine),



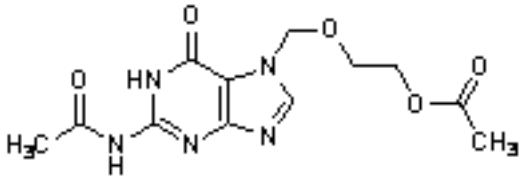
J. 9,9'-[ethylenebis(oxymethylene)]bis(2-amino-1,9-dihydro-6*H*-purin-6-one) (9,9'-[ethylenebis(oxymethylene)]diguanine),



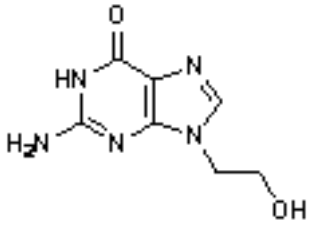
K. 2,2'-(methylenediimino)bis{9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one} (N^2,$N^{2'}$-methylenediaciclovir),



L. *N*-(9-acetyl-6-oxo-6,9-dihydro-1*H*-purin-2-yl)acetamide (N^2,9-diacetylguanine),



M. 2-[(2-acetamido-6-oxo-1,6-dihydro-7*H*-purin-7-yl)methoxy]ethyl acetate,



P. 2-amino-9-(2-hydroxyethyl)-1,9-dihydro-6*H*-purin-6-one (9-(2-hydroxyethyl)guanine).